Optogenetic Stimulation of Peripheral Vagus Nerves using Flexible OLED Display Technology to Treat Chronic Inflammatory Disease and Mental Health Disorders

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Abstract: *This work explored the viability of a biophotonic alternative to conventional prescription-drug-based treatments for inflammatory disease and mental health disorders using a non-invasive drug-free optogenetics-based therapy that treats patients by optically stimulating afferent branches of the auricular vagus transcutaneously via the outer ear using a high-resolution, addressable array of organic light emitting diodes (OLEDs) manufactured on a flexible plastic substrate. Preliminary analysis and optical measurements indicate that our 620 nm flexible red OLED display technology is bright enough to induce therapeutic optical stimulation in optogenetically modified neural tissue.*

Keywords: Optogenetics; neuromodulation; organic light emitting diode; OLED; TFT; vagus nerve stimulation.

Introduction

As an alternative to conventional prescription-drug-based treatments, direct neuromodulation of peripheral nerves promises drug-free treatment of chronic inflammatory disease and mental health disorders affecting civilians, military personnel and veterans, including arthritis, systemic inflammatory response syndrome, inflammatory bowel disease, post-traumatic stress disorder (PTSD), anxiety, and depression [1, 2]. For treating depression, peripheral neuromodulation does not have the side effects or drug interactions associated with prescription antidepressants and is potentially more effective [3]. For patient treatment, existing peripheral neuromodulation-based therapies typically stimulate afferent vagus nerves using an implanted electrical transducer [4, 5]. However, most of these devices are still only used as a last resort, because they are large and often induce undesirable physiological side effects, along with requiring invasive surgery for placement [2].

One new non-invasive approach is the transcutaneous electrical stimulation of the auricular branches of the vagus using an electrical transducer attached to the surface of the outer ear [6]. The auricular vagus nerve is targeted because human anatomical studies have identified the outer ear as the only location on the human body where afferent vagus nerves are accessible transcutaneously [7]. However, both the transcutaneous and implanted transducers used in existing electrical stimulation-based devices are quite large (>1 cm) in relation to the size of individual nerve fibers or branches (<<1 mm). Hence, they lack the precision required to activate specific or individual afferent vagus nerve fibers in the outer ear, each of which provide specific pathways to a localized region of the brain. Unfortunately, simultaneously stimulating all branches of the vagus in the outer ear can cause undesirable physiological side effects by also simultaneously stimulating additional portions of the brain not associated with the disease being targeted. These side effects can include changes in voice/speech, throat/larynx spasms, alterations of cardiac output and/or pulmonary ventilation, as well as difficulty swallowing [8].
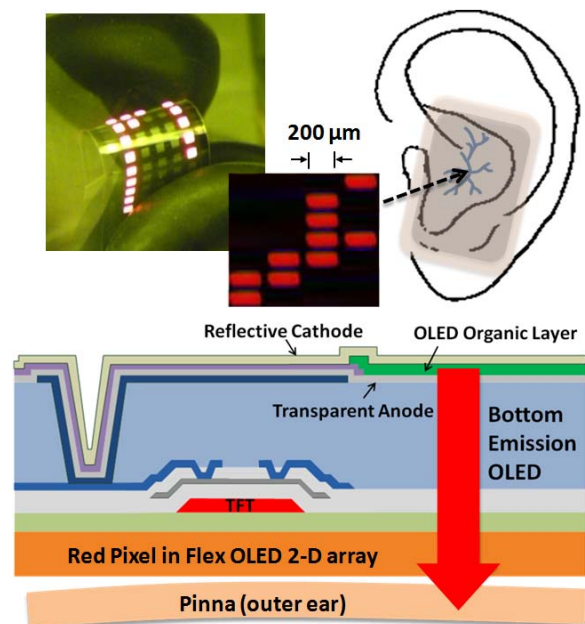

Figure 1. Concept for a transcutaneous auricular vagus nerve optogenetic neurostimulator using flexible red OLED display technology to treat mental health disorders and chronic inflammatory disease using a disposable biophotonic smart bandage applied to the patient's outer. A reconfigurable, two-dimensional, optical stimulus pattern of activated red OLED pixels targets specific auricular branches of the vagus nerve while not stimulating neighboring off-target afferent nerve fibers to minimize side effects

As a solution to these limitations and as an alternative to electrical vagus nerve stimulation (VNS), we introduce a notional Optogenetics-based approach to *optically* stimulate *individual* or localized afferent auricular vagus nerve branches in the outer ear using a high-resolution, two-dimensional (2-D), addressable array of red organic light-emitting diodes (OLEDs) manufactured on a thin and flexible plastic substrate. Optogenetics is a relatively new neuromodulation technique that uses light to optically stimulate neural tissue [7]. However, existing Optogenetic therapies typically require extremely invasive surgery to either place a fiber optic probe deep within the brain or attach an optical transducer directly to the surface of the brain [9]. In this work, we've focused on exploring the viability along with describing the underlying flexible display technology for a new *non-invasive* Optogenetic therapy that uses a flexible skin-mounted optical (OLED) transducer, that unlike existing invasive Optogenetic approaches, may offer a viable path for future clinical use [10].

New Approach using Flexible OLED Display Technology

As illustrated in Fig. 1, our conceptual configuration is a flexible OLED display-based array, similar to a peel-and-stick adhesive bandage that can be applied directly to the surface of the outer ear. For treatment, auricular vagus nerves in the patient's pinna would be genetically modified to respond when illuminated by incident red light from the conformal 2-D OLED array. During treatment, individual red OLED pixels in the flexible display, sized between 100 μm to 1 mm, would be activated (addressed) to target and selectively illuminate specific afferent auricular vagus nerve branches. As illustrated in Figure 1, our assumption is that the optimum reconfigurable optical stimulus pattern would illuminate most of one particular auricular vagal nerve branch, and not illuminate (i.e., optically stimulate) neighboring off-target vagus nerves branches to provide the desired specificity and minimize unwanted physiological side effects during treatment.

Low Cost Mass Production Technology

The notional transcutaneous VNS OLED 'bandage' would be manufactured on a thin plastic substrate using commercial thin-film, flexible-display technology. Flexible displays are fundamentally a very thin, transparent sheet of plastic, approximately the same thickness as a piece of paper, and are constructed by sequentially layering and patterning a series of nanoscale thin films. This is a technology that we're extremely familiar with and we've already manufactured and demonstrated a number of flexible full-color OLED display prototypes using our pilot line at the Arizona State University Flexible Electronics and Display Center. (Figure 2) [11-13].

Our Optogenetic VNS stimulator concept is designed to leverage the immense mass production capabilities of the

Figure 2. Flexible 7.4" diagonal Flexible OLED display manufactured on a 370 mm x 470 mm gen2 plastic substrate at the Arizona State University Flexible Electronics and Display Center (FEDC).

commercial display industry, which is currently transitioning from rigid glass substrates to flexible plastic substrates for mobile devices. Given that several billion drug prescriptions are filled annually in the USA, we believe that a low-cost, mass-production technology is required for our concept to be commercially viable. We envision manufacturing the OLED-based optogenetic VNS stimulators on Gen4 (730×920 mm) or larger, multi-device, flexible substrates that can then be cut up into much smaller individual disposable devices. For perspective, flat-panel displays were manufactured at a rate of 100 square kilometers per year in 2012, which is enough material to cover the island of Manhattan [14]. If just 1% of existing flat-panel industrial capacity were diverted to manufacture flexible biophotonic devices, approximately one billion (~ 10 cm^2) devices could be manufactured annually.

Preliminary Experimental Results and Analysis

High Brightness Flexible OLED Display Technology: The flexible bottom-emitting OLEDs used in this work were fabricated on patterned indium-tin oxide (ITO) polyethylene naphthalate (PEN) transparent substrates. The 5 mm^2 OLED test structure consists of a 10nm layer of hexaazatriphenylene hexacarbonitrile (HAT-CN) hole injection layer (HIL), followed by a 40nm 4-4'-bis[N-(1-naphthyl)-N-phenyl-amino]biphenyl (NPD) hole transport layer (HTL). 10-40 nm of red and blue emissive layer (EML) was deposited onto the HTL. Red comprised a co-host structure of phosphorescent red dopant, and Blue consisted of single host:fluorescent blue dopant. Next, a 10-30nm thick hole blocking layer (HBL) was deposited onto the EML, followed by a 30nm electron transport layer (ETL) consisting of doped 8-hydroxyquinolinolato-lithium (Liq). A 2nm Liq electron injection layer (EIL) was deposited onto the ETL, followed by a 100nm layer of MgAg or Al cathode metal. Films were deposited by vacuum thermal evaporation in a Sunicel Plus 400 system made by Sunic Systems at pressures below 5x10-7 Torr. Films were patterned using metal shadow masks with no break in vacuum between layers. The flexible OLED
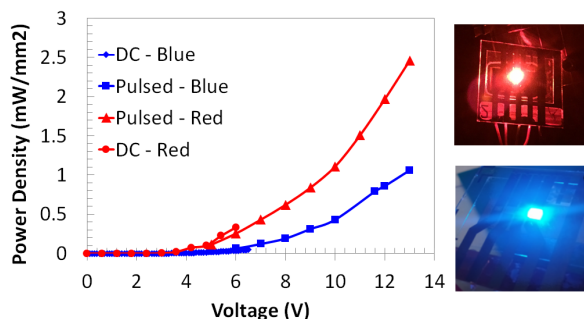
Figure 3. Red and Blue Flexible OLED test structure intensity as a function of forward bias voltage

devices were then encapsulated using a thin-film barrier material from 3M Company. Power density/voltage measurements were made using a Keithley 2400 source meter, and silicon photodiode 818-UV from Newport.

To determine the initial viability of our concept, we first needed to determine whether our flexible OLEDs can emit a light bright enough to induce optical stimulation in (opto)genetically modified neural tissue. Kim, et. al. previously reported that a minimum of 1 mW/mm^2 of instantaneous pulsed irradiance at a wavelength of ~450 nm (blue) is required to induce optical stimulation in ChR2 expressing neurons, which are commonly used in optogenetics research [15]. While a close match to the optical stimulation wavelength, the instantaneous light intensity of 1 mW/mm^2 is ~10X greater than the intensity of our existing 455 nm blue OLEDs biased at 7 volts DC, which is a typical DC bias in commercial OLED displays [11]. Initial attempts to improve light intensity by increasing the DC bias above 7 volts significantly degraded the OLED organic emission layers due to current induced, localized joule heating. As reported earlier [9], we discovered that pulsing the OLED power supply at 20 Hz, along with attaching a thin and flexible metal foil cathode heat sink, allowed us to increase the OLED bias voltage to 13 volts. This provided a 10X boost in instantaneous blue light intensity from 0.1 mW/mm^2 (under DC bias) to 1 mW/mm^2 (pulsed-mode) without degrading or damaging the OLED organic layers (Figure 3).

In Vitro Optical Stimulation using Blue OLEDs: Using this new method to significantly boost emitted OLED light intensity, our initial focus was to determine whether pulsed blue light from an OLED test structure was able optically stimulate cortical neurons *in vitro* using cells expressing the opsin ChR2-YFP. For this work, primary cortical E18 neurons were cultured on the surface of a conventional transparent microelectrode array (MEA). The 5 mm^2 blue OLED test structured was then

positioned directly underneath the MEA and activated to optically stimulate the cultured neurons using 20 Hz, 1 mW/mm^2 pulsed 455 nm blue light (Figure 4a.). Electrical activity of the blue OLED photostimulated cortical neurons was monitored and evaluated for correlation with the incident 20 Hz light pulse. As illustrated in Fig. 4b and 5, the detected spike rate in the neural tissue expressing ChR2-YFP increased when the blue OLED was turned on, and then immediately decreased to baseline values when the blue OLED was turned off. As evident in the figures, while present, the observed changes when the blue OLED was turned on and off were not especially pronounced, which we attribute to a transfection efficiency of only about 5%, which needs to be addressed in future work.

Optical Stimulation of the Vagus using Red OLEDs: However, for our conceptual VNS application, red light is preferred to maximize the penetration depth of the excitation light source into the tissue of the outer ear as opposed to blue or green light, which will be absorbed more superficially. The cartilage in the outer ear is non-innervated, avascular tissue, indicating that the auricular vagus branches would be as superficial and approximately the same depth as the dermal layer of the pinna, setting the maximum depth of the auricular vagus nerves to about 2 mm, which is comparable to the penetration depth δ of 625 nm red light in human skin, where the incident (red) light intensity is approximately reduced by a factor of 1/e.
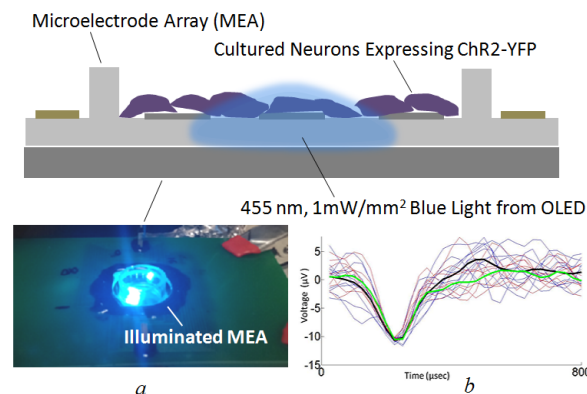

Figure 4. (a) Experimental test configuration with 32 channel microelectrode array (MEA) connected to Intan neural recording system used to demonstrate 1 mW/mm^2 of pulsed 455 nm light from blue OLED test structure can optically stimulate cultured neurons *in vitro* using cells expressing ChR2-YFP (b) Time aligned neural spike activity under blue OLED optical stimulation. Green and black waveforms are the mean values of all recordings captured before (green waveform) and after (black waveform) optical stimulation.
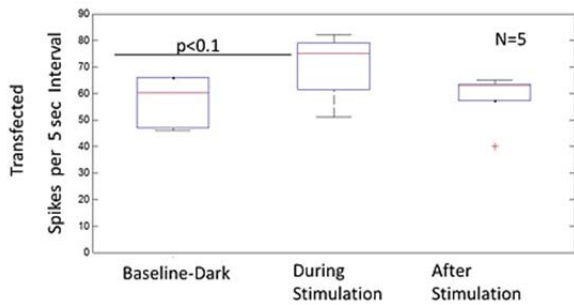
Figure 5. Optical stimulation data for primary cortical E18 neurons in culture transfected with ChR2-YFP gene shows a statistically significant increase in spike rate when illuminated by pulsed 1mW/mm^2 455 nm light from the 5 mm^2 blue OLED test structure

Klapoetke, et. al. [16] recently reported the ability to induce (optogenetic) stimulation in transfected neural tissue with optical powers as low as 0.015 mW/mm^2 using the red-shifted opsin Chrimson and a 617 nm red light source. Hence, if vagus branches could be transfected with this new opsin, it may be possible to induce optical stimulation using incident red light intensity as low as ~0.04 mW/mm^2 [0.015 mW/mm^2 ÷ 1/e]. Our existing red OLEDs reach a peak emission at ~620 nm [11], which provides an excellent match for the red-shifted Chrimson opsin [16]. Using 13 volts (pulsed − Fig. 3), we would be able to deliver approximately 2.5 mW/mm^2 of red light to the surface of the outer ear, which is >50X the estimated/calculated power required to induce optogenetic stimulation in neural tissue modified to express the Chrimson opsin, indicating that our notional concept appears viable.

Conclusions
This work explored the initial viability of a new drug-free non-invasive optogenetic technique to treat chronic disease by optically stimulating selected afferent auricular vagus nerve branches via the outer ear using a high-resolution, 2-D addressable array of OLEDs manufactured on a thin and flexible plastic substrate.

Acknowledgements

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